Gastro-intestial microflora of laboratory mice

Koopman, Johan Pieter

SUMMARY

The research described in this thesis with mice aims to be used as a possible starting point for the development of highly standardized experimental animals. In standardization of experimental animals, the microflora should be involved for various reasons as presented and discussed in the introduction. In that chapter the close relation between the microflora of the gastro-intestinal tract and the normal physiological processes of the host are discussed. Not only the intestinal physiology, such as bile acid metabolism, mucosal renewal and intestinal peristalsis, but also general physiological processes such as the circulation, the immune system and even the basal metabolism of the host appeared to be involved. These considerations have been the basis upon which the need for the presented studies was founded.

Biomedical studies of all kinds of physiological processes require homogeneous laboratory animal populations. It was realized that this means that in some experiments the animals used should not only be genetically homogeneous, and of the same age, sex and weight but also with regard to the composition of their flora.

Since the anaerobic fraction of the intestinal microflora appeared most closely involved in these functions, optimal circumstances for anaerobic culturing were

realized in a specially designed anaerobic chamber (Chapter 2). How far these anaerobic conditions had to go was investigated in the mouse and rat cecum *in vivo* (Chapter 3). In artificial culture media a comparably low oxidation-reduction potential was found and maintained.

With this special equipment a start was made with the isolation of strict anaerobic species from the cecum of the mouse (Chapter 4). These was subsequently either alone or in different combinations administered to germ-free mice for association (Chapter 5).

In as much as it was not primarily our intention to investigate whether these associations were possible, but more whether they were involved in the various flora-influenced physiological processes, a choise had to be made for the most suitable tests to investigate the achieved level of normalization. For practical reasons, in this selection a particular process that was strongly influenced by certain anaerobic species played a major role. It concerns the process which limits colonization of the digestive tract by 'foreign' potentially pathogenic species, and which is called the *'Colonization Resistance* (CR) of the digestive tract. Direct or indirect tests for the CR were considered preferable because a sufficient CR would strongly help to realize our major aim to keep a certain flora as constant as possible. The tests needed to estimate the degree of normalization after association of

germ-free mice with certain combinations of anaerobes were consequently directly or indirectly focussed on the CR. Much emphasis was given to the question whether the gastro-tro-intestinal tract transit time, which could be relatively easily determined, correlated with the CR (Chapter 6 and 7). Since the intestinal contents in rodents consist apart from soluble substances for most part of particulate matter, the passage time was initially determined with minute stainless steel balls given intra-gastrically. Because of the limited applicability of this test in animals with a reduced CR, additional observations have been made with a dye (carminic acid), which could be recovered from the feces with great accuracy (Chapter 8). Both techniques have led to the conclusion that in mice the gastro-intestinal transit time fluctuates rather strongly from day to day. This implied that it could not be used in individual germ-free animals for investigation of the degree of 'repair' of the transit time following association with a certain mixture of anaerobic bacteria. However, we found indications that the transit time, determined in groups of mice, was rather suitable to estimate the CR of the digestive tract.

The CR, determined by experimental oral contaminations with *E.coli* in individual mice, appeared well reproducible (Chapter 6), but strongly dependent on the present flora (Chapter 5). It appeared also that these para-

meters changed in the same way as the cecum weight after association of germ-free animals with microfloras of increasing complexity. No correlation was found between the value of the CR and certain environmental factors in the gut such as pH and oxidation-reduction potential. The effect of certain floras on the host can be estimated and compared with the results obtained in the direct and indirect tests for the CR.

Finally, a number of possibly practical implications are presented for the start of animal colonies with a defined flora (Chapter 9).